THEODORE E. WOODWARD AWARD

THE EVOLUTION OF OBESITY: INSIGHTS FROM THE MID-MIOCENE

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ABSTRACT

All humans are double knockouts. Humans lack the ability to synthesize vitamin C due to a mutation in L-gulono-lactone oxidase that occurred during the late Eocene, and humans have higher serum uric acid levels due to a mutation in uricase that occurred in the mid Miocene. In this paper we review the hypothesis that these mutations have in common the induction of oxidative stress that may have had prosurvival effects to enhance the effects of fructose to increase fat stores. Fructose was the primary nutrient in fruit which was the main staple of early primates, but this food likely became less available during the global cooling that occurred at the time of these mutations. However, in today's society, the intake of fructose, primarily in the form of added sugars, has skyrocketed, while the intake of natural fruits high in vitamin C has fallen. We suggest that it is the interaction of these genetic changes with diet that is responsible for the obesity epidemic today. Hence, we propose that Neel's thrifty gene hypothesis is supported by these new insights into the mechanisms regulating fructose metabolism.

Key Words: uricase, vitamin C, ascorbate, obesity, metabolic syndrome

In 1962, the anthropologist James Neel proposed that the genetic adaptation of our human ancestors to famine might have preset modern day humans to have an increased risk for obesity and diabetes when foods became more plentiful (1). While the hypothesis was initially well received, the inability to identify the specific genes potentially driving this response has reduced enthusiasm for the hypothesis.

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Potential Conflicts of Interest: Dr. Johnson has one patent and several pending patents related to uric acid management in patients with hypertension and cardiovascular disease.

Furthermore, based on equations developed by Haldane (2), it has been argued that if the genetic changes occurred more than 2 million years ago (Ma) that everyone should be carrying the genes yet not everyone is obese (3). Hence, this has led a number of investigators to question the validity of this hypothesis.

In this brief paper, we revisit Neel's hypothesis and make the case that our ancestors underwent a major genetic adaptation not once, but at least twice, during our evolution and that the primary change involved mutations that silenced genes in vitamin C synthesis and uric acid degradation, respectively. In effect, all humans are double knockouts, and this resulted from mutations that occurred in the Eocene and Miocene, respectively. We have hypothesized that it is the functional loss of these genes, not the gain of prodiabetic genes, that provided a key role in survival in ancestral times. Furthermore, these mutations do not cause obesity in themselves, but rather increase the susceptibility for obesity and diabetes in response to specific food groups, and that it is the genetic and environmental interaction which is driving the epidemic of obesity and diabetes today. Ironically, despite the general belief that survival is associated with increased antioxidant capacity (4), we suggest that it was the opposite, the ability to increase oxidative stress, that was associated with survival among early hominoids.

THE "URICASE KNOCKOUT" HUMAN

Uric acid is a metabolic product of purine metabolism and is generated from xanthine by the enzyme xanthine oxidoreductase (XOR). In most mammals uric acid is degraded to allantoin by the enzyme uricase (urate oxidase), resulting in relatively low serum uric acid levels in the circulation (0.5 to 2.0 mg/dl). Humans and the great and lesser apes, however, lack uricase and as a consequence have higher serum uric acid. Genetic studies have suggested that all hominoids (apes) initially had a progressive loss of uricase activity due to mutations in the promoter region, but that distinct silencing mutations occurred in the great ape/human clade and the lesser ape clade at approximately 15 million years ago and 9.8 Ma, respectively (5), Studies in old world monkeys have also documented a reduction in hepatic uricase activity compared to other mammals (6), and some new world monkeys have elevated serum uric acid suggesting they had independent mutations in uricase (7). The parallel nature of the mutations suggests that the loss of uricase was associated with a general natural selection advantage for primates living in the Miocene (23 to 5 Ma).

The consequence of a loss of uricase is an increase in serum uric acid. Studies performed at the San Diego zoo documented serum uric acid levels in the 2.5 to 3.5 mg/dl range among apes lacking uricase (8). Similarly, we found that uric acid levels among Yanomamö Indians living under their native conditions had serum uric acid levels in a similar range (Figures 1 and 2) (9). This suggests that the serum uric acid that occurred in the early hominoids undergoing the mutation was likely in the 2 to 4 mg/dl range.

INTERACTION OF URICASE MUTATION WITH DIET

In contrast, serum uric acid levels are higher in western societies which can be partially attributed to western diet. For example, gout, which is a surrogate marker for a high uric acid, emerged as a common ailment among the wealthy and typically obese individuals in 18th and 19th century England. Gout prevalence has continued to increase in Europe and the United States over the last century. Serum uric acid levels have also been rising, with mean levels >5.5 mg/dl in women and >6.0 mg/dl in men today (8). Indeed, it is not uncommon for individuals to have serum uric acid levels >8.5 mg/dl.

Similarly, the prevalence of gout was low in most indigenous populations before the introduction of western diet, including among the Australian aborigine, the Maori Indian, and most native peoples (8). However, after introduction of the western diet, a marked rise in serum uric acid level with increased prevalence of gout was observed.

FIG. 1. James V. Neel drawing a blood sample from a young Yanomamö Indian (42). Dr Oliver (author) and Dr Neel performed several studies of the Yanomamö Indians that live in the jungles of southern Venezuela. In particular, this is an indigenous people with normal blood pressure and a diet high in potassium and low in sodium (42).

FIG. 2. William J. Oliver with Yanomamö children.

Hyperuricemia and gout were also uncommon among rural communities, but increased markedly following the migration of individuals to cities or to communities where western diet was dominant (8).

The reason why higher serum uric acid levels are observed in western societies could relate in part to the diet. Purines have historically been considered the major source of uric acid, but another common source is fructose. Fructose is a simple sugar that is present in fruit and honey but which is also present in table sugar (sucrose), a disaccharide that contains equal amounts of glucose and fructose. In the United States, another major source of fructose is high fructose corn syrup (HFCS), which is a sweetener generated by the enzymatic degradation of corn syrup and which commonly occurs in the ratio of 55% fructose and 45% glucose. Whereas purines raise uric acid by increasing substrate (xanthine, Figure 3), fructose increases uric acid as a consequence of the unique metabolism of fructose in which transient ATP depletion commonly occurs with the generation of AMP (10). AMP deaminase is also stimulated, resulting in the metabolism of the nucleotide breakdown products to uric acid, which rises both intracellularly and in the circulation (11).

Given that both high purine (usually high protein) and high fructose diets are associated with increased risk for hyperuricemia and gout (12, 13), the question arises as to which of these dietary items might be contributing to the rise in serum uric acid that has been observed over the last 100 years. In this regard, there has been a progressive reduction in protein intake in the United States in the last several decades (14). In contrast, there has been a remarkable increase in the intake of added sugars, from a mean of 4 pounds of sugar intake per year in 1700

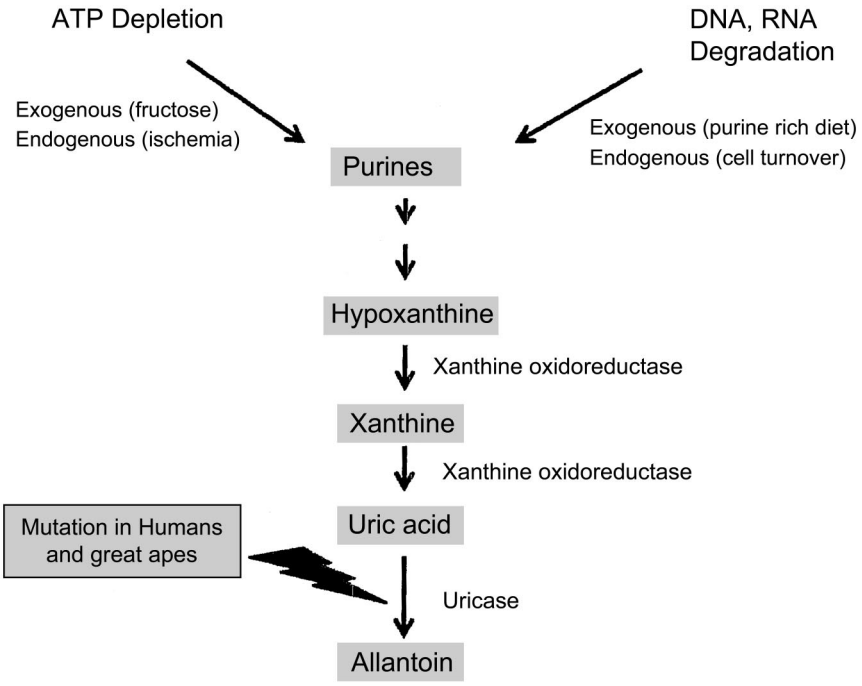

FIG. 3. Major pathways for generating uric acid.

in the United Kingdom and United States, to greater than 150 pounds per year today (15). Among some populations, the intake of added sugar is even higher. For example, one study of 8th graders showed that 40% of the diet was from sugar. In the NHANES (2003–2006) study, we found that 25% of the population was ingesting over 130 g of fructose per day, which equates to over 200 pounds of sugar per year (16). Levels this high were also observed in more than 50% of obese subjects from Mexico City in a recent clinical study (M. Madero, personal communication). Thus, some individuals are ingesting more than 50 times the mean amount of sugar than was being ingested just 300 years ago. In turn, the increase in fructose intake has been found to be associated with an elevation in serum uric acid in most (13, 17) but not all (18) studies.

URIC ACID: A SURVIVAL FACTOR FOR EARLY HOMINOIDS?

The uricase knockout has been calculated to have occurred in the mid Miocene, approximately 15 Ma. This was a period of time when our early hominoid ancestors were living primarily in Europe, Asia, and

Africa. Most evidence suggests that the early hominoids were living in tropical rain forests and that the primary staple was fruit, for which the primary energy constituent is fructose. During the mid-Miocene there was global cooling that adversely affected local fauna and flora, and this resulted in a loss of tropical rain forests with their replacement by deciduous forests, particularly in Eurasia. The development of seasonal climates resulted in periods of periodic food shortage in Europe, resulting in the progressive reduction in the primate population with their concentration to a few geographically isolated habitats. By the late Miocene there is no longer evidence for hominoids living in Europe. However, the palaeoanthropological record suggests that an ape from Europe may have migrated back to Africa to become the ancestor of the modern great apes and humans (19, 20). We have hypothesized that it was during the excursion to Europe that the uricase mutation occurred, and that the combination of marked survival benefit, coupled with a small and geographically isolated population, allowed its rapid emergence and universal replacement in the small surviving population (21). How would a uricase mutation provide survival benefit to early hominoids?

Recent studies suggest that fructose may not be simply an energy source, but may have specific metabolic effects that may aid in increasing fat stores (15). Thus, if laboratory animals are pair fed fructose or glucose, only the fructose fed rats develop marked hypertriglyceridemia and fatty liver (22). Furthermore, fructose fed rats will also develop intraabdominal fat accumulation, insulin resistance and elevated blood pressure, even if overall energy intake is restricted (23, 24). These alterations are similar to what is observed in hibernating mammals prior to hibernation, and likely provides a means for survival during a period of food shortage (25). Interestingly, vitamin C is known to partially block the effects of fructose to induce this metabolic phenotype (26). Importantly, as fruit ripens towards the end of the summer season, the vitamin C content falls whereas fructose content increases (27). Hence, fruits are an important food source not only for their energy intake, but for their metabolic effects to increase fat stores. In addition, fruits are the most lipogenic at the end of summer, prior to seasonal cooling and a reduction in food availability.

The absorption of fructose in the intestine is largely mediated by Glut5 followed by uptake via Glut2 into hepatocytes where it is metabolized by fructokinase. Levels of these transporters as well as fructokinase are known to be markedly variable in humans and other species. In this regard, we have recently found that uric acid is a marked regulator of Glut5, Glut2, and fructokinase expression in a

wide variety of cell types. Indeed, acutely raising uric acid in rats by administering a uricase inhibitor results in a marked increased expression of Glut2, Glut5, and fructokinase in a variety of organs (Sánchez Lozada LG et al, manuscript in preparation). The mechanism appears to be due to the prooxidative effects of uric acid within the intracellular environment. Indeed, while uric acid is an antioxidant in the extracellular setting, recent studies suggest that inside the cell, uric acid acts as a prooxidant by stimulating NADPH oxidase (28). Thus, uric acid may have a key role in increasing the sensitivity of animals to the metabolic effects of fructose. Consistent with this finding, the development of obesity and metabolic syndrome in rats in response to fructose can be largely prevented by lowering uric acid (22).

More recent studies suggest that uric acid may have a direct effect independent of fructose on weight gain and fat accumulation. Specifically, uric acid causes mitochondrial dysfunction with specific effects to increase fat accumulation by both increasing fat synthesis and by blocking fat oxidation (Sánchez Lozada LG et al, manuscript in preparation). Indeed, acutely raising serum uric acid with a uricase inhibitor in rats will result in fat accumulation in the liver within 24 hours, and this is not observed if the rise in uric acid is prevented. Other studies have suggested effects of uric acid on blood pressure, insulin resistance, and adipocyte activation (28, 29). Indeed, Cheung et al recently reported that mice that cannot produce uric acid (XOR knockout mice) have a central defect in adipogenesis and fail to get fat (30).

THE "VITAMIN C SYNTHESIS KNOCKOUT" HUMAN

All humans have also lost the ability to synthesize vitamin C (ascorbate). This is due to a mutation in L-gulono lactone oxidase that is thought to have occurred during the late Eocene, approximately 30 to 40 million years ago (31, 32). The mutation involved all primates except prosimians.

Ascorbate has multiple functions but one of its major actions is as an antioxidant. Interestingly, it also has a role in blocking fructose metabolism. Thus, as mentioned earlier, antioxidants including vitamin C are known to partially block the effect of fructose to induce the metabolic syndrome in laboratory animals (26). Vitamin C also lowers uric acid by stimulating urate excretion (33). Similarly, fructose inhibits vitamin C synthesis in mammals that are capable of synthesizing vitamin C (34). Hence, vitamin C may be considered an antidote to the effects of fructose to stimulate fat storage. Not surprisingly, vitamin C

levels mirror in opposition changes in uric acid that occur in the hibernating mammal (9).

The loss of vitamin C synthesis occurred during the Eocene at a time when there was global cooling and a significant extinction of mammalian species (35, 36). Hence, we have hypothesized that the mutation of L-gulono lactone oxidase may have been of benefit to early primates by increasing uric acid levels and enhancing fructose effects (37). In other words, the mutation may have worked via a parallel mechanism as proposed for the uricase mutation.

PARALLEL MECHANISMS IN BIRDS

It is interesting to note that birds also lack uricase and also secrete urate. Some lineages of perching birds (passerines) have also lost the ability to make ascorbate, while others have not (38). Since a requirement for ascorbate is universal, we presume that birds that have lost the ability to make ascorbate have access to dietary ascorbate.

This occasional loss of ascorbate in a background genetic system that lacks uricase is the mirror image of the situation in primates, where uricase was occasionally lost in a genetic background that lacks ascorbate. This suggests that the subset species of these birds that gain access to ascorbate from sources that also deliver large amounts of fructose might display analogous biochemical physiology to that described here for mammals.

A REVALUATION OF PRIOR HYPOTHESES RELATED TO VITAMIN C AND URIC ACID

In the mid 1950s, Harman et al proposed that oxidative stress might be largely responsible for aging (39). In this regard, the loss of ascorbate synthesis by primates in the Eocene was a mystery, since ascorbate is such a powerful antioxidant. Pauling suggested that the mutation may have resulted as a consequence of lack of need (40), since primates were ingesting large amounts of exogenous vitamin C present in fruits, which was considered the dominant food source. However, an accidental mutation of vitamin C synthesis could not account for why this mutation is expressed by all primates (except prosimians), as the latter requires a natural selection advantage to replace the prior genotype.

The major hypothesis that has been used to explain the uricase mutation was proposed by Ames et al, who suggested that uric acid is a powerful antioxidant, and that the mutation occurred in response to the prior mutation in vitamin C synthesis (4). According to this hy-

pothesis, the lack of vitamin C resulted in increased oxidative stress that had effects on both aging and cancer, and the mutation of uricase resulted in an increase in uric acid that could provide key antioxidant activity. As mentioned, uric acid is a potent antioxidant that can block oxidants *in vitro* and in the extracellular environment (4). However, as discussed above, while uric acid is an antioxidant in the extracellular setting, once it enters the cell it activates NADPH oxidase and causes oxidative stress (28, 41). Furthermore, we have found that the prooxidative effects of uric acid may be responsible for how uric acid alters fructose metabolism.

CONCLUSION

All humans lack a key enzyme in vitamin C synthesis and in uric acid degradation due to genetic mutations in L-gulono lactone oxidase and uricase, respectively. Both mutations occurred during a period of global cooling associated with a shortage of food supplies, and recent studies suggest that these mutations may have had a survival advantage by augmenting the effects of fructose to increase fat stores. Interestingly, these prosurvival effects were mediated by increased oxidative stress. However, the introduction of sugar and high fructose corn syrup in the current American diet has skyrocketed, with a nearly 30- to 50-fold increase in fructose intake since 1700 (15). We believe that these dietary changes, coupled with the presence of our double knockout genotype, is largely responsible for the obesity epidemic today.

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We dedicate this paper to the memory of James V. Neel who was an example of the exemplary anthropologist and whose studies crossed from evolution and physical anthropology to the general field of medicine.

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DISCUSSION

Weir, Baltimore: Rick, as always, I wish you had more time. The issue of fructose in the diet obviously is very important today, given the frequency of it certainly in the soft drinks that our kids eat and everything else. I guess my questions is, we focused on the conversation about uric acid in isolation, yet have not delved into other factors in the diet which could be important. You didn’t really get into it, but you alluded to the fact that this type of syndrome is associated, for example, with increasing sensitivity to dietary salt; and of course the question is, is it more salt or less potassium; and of course there are newer studies indicating, for example, that animal protein versus vegetable protein may also be more likely to induce, you know, incident hypertension. So, I guess my question is, in a nutshell, is this fructose alone or is it fructose in conjunction with maybe

higher sodium and less potassium and more animal protein that may be part of the problem?

Johnson, Aurora: Well, fructose is just one factor, and clearly there are many other factors that are driving the cardiovascular disease epidemic; but we think fructose is a major factor that drives the metabolic syndrome. Having said that, we think that whatever raises uric acid can also induce these similar features. So, for example, the second best way to raise uric acid is to drink beer, because of the yeast in beer, which contains RNA; and we believe that the beer belly is a *forme fruste* of metabolic syndrome, because you often have hypertriglyceridemia, hypertension and so forth as an association with that. So, we do think that this is a central pathway, but we also, you know, acknowledge the improtance of salt and other mechanisms and high saturated fat diets and its effects on cholesterol, for example. So, I mean, it's not the only mechanism. So you're right.

Weir, Baltimore: Do you think also the animal protein story also may be related?

Johnson, Aurora: So, it turns out that animal protein and vegetable protein have different amounts hypoxanthine and xanthine in them. We believe that that's more important than the purine content.

Weir, Baltimore: Thanks.

Ende, Philadelphia: Fascinating subject and I think your work adds to the growing body of epidemiologic research associating hyperuricemia with cardiovascular risk. With that in mind, what is the status of work on uric acid lowering therapy as a protective factor for cardiovascular outcomes?

Johnson, Aurora: Well, there is another NIH trial that has just been completed in which probenecid, allopurinol and placebo are compared in obese, pre-hypertensive adolescents. There was a very dramatic effect on blood pressure with both drugs. Also, there was a benefit on weight. The paper is being prepared by Dr. Feig, and I will say the NIH has invited us to come up with a multi-center trial to see if lowering uric acid can help prevent metabolic syndrome and hypertension in pre-hypertensive individuals.

Billings, Baton Rouge: I found that your discussion was provocative along the lines that there has been some suggestion in African Americans, those that survived the trip across in the slave ships, had a genetic trait that allowed them to retain salt and, therefore, retain water and, therefore, survive; and it's been suggested by some, and I don't know whether the gene has been identified, that this type of gene allowed their survival but also now is very important in the fact that African Americans have a significant amount of hypertension. How does this relate to what you were telling us?

Johnson, Aurora: Well it's possibly true. That is another hypothesis. It's also true that the African American in this country eats a lot more sugar than the Caucasian, and this goes way back to the days on the plantations; and you can find evidence for increased sugar intake and higher uric acids among African Americans going way back. Now interestingly, there was a time when African Americans had a lower rate of diabetes than Caucasians, if you go back into the 1800s; and so, you know, it's kind of an interesting thing, and Africans in Africa have had a lower rate of obesity and diabetes until just recently, where now, we are starting to see it in the urban communities in association with sugar intake. So, I keep relating it to sugar intake and uric acid, but it is quite possible there could be genetic polymorphisms also linked to this, and it could be related to that hypothesis.

Schreiner, San Francisco: So Rick, the very interesting implications of this, of course, are that in a setting of seasonality where you would have transient exposure to fructose, say at the end of the growing season, then it's in your biological interest to be able to maintain your blood pressure, add triglycerides, put on fat stores; and the difference between then and now is a lack of seasonality. High concentrate, continuous

exposures to lots of fructose. I am wondering if there is any evidence on the animal side—the ones that have uricase, in the settings where, in fact, hibernation that often is preceded by consumption of high fructose entities, the way bears eat berries, for example. It there any evidence for physiological regulation of uricase that says that of animals that need to become fat and inactive, that they can actually modulate the system in a way that's reversible, unlike the mutation that you're saying allowed this arrival of hominid precursors to man?

Johnson, Aurora: Yeah, that's a very exciting topic. I actually have been studying the thirteen-lined ground squirrel, which is a hibernating squirrel. I am doing some studies with Sandra Martin. These animals, right before they hibernate, become hyperuricemic. They develop all features of hypermetabolic syndrome. They get insulin resistance, hypertriglyceridemia, fatty liver—the whole bit, and they do this during the fall when they are eating and accumulating fat, and then as soon as they hibernate, they plummet their uric acid levels to almost zero, and it's associated with knocking xanthine down to almost zero. So they're turning off the enzyme proximal to xanthine, and we think its adenosine deaminase; and then AMP levels go up, and AMP kinase gets activated, and the uric acids stay low. These animals are hibernating at four degrees, but they have no GFR and the uric acid starts to build up because they are making a tiny bit, but over time, it starts to accumulate. After two weeks at four degrees, they suddenly warm up to 37 degrees but stay asleep, and then, uric acid plummets, and allantoin goes way up; and I looked it up, and uricase is inactive under 20 degrees. So what happens is these animals, we believe, have developed a system to hibernate, but they have to warm up every two weeks for two days so that they can plummet their uric acid and activate AMP kinase. So, it's a very exciting story. We are in the process of trying to prove various aspects of it, but this is a very fascinating story. There are other parallels in different ways of how different animals have utilized the same pathway. We knocked out uricase, but there are other ways that animals have become obese. You and I can talk about it afterwards.

Abboud, Iowa City: Very interesting. I noticed that you have a pattern. Could that possibly be trying to develop a pattern? Could that possibly be for uricase as a therapeutic antihypertensive?

Johnson, Aurora: Well, you know when we discovered that lowering uric acid lowered blood pressure, and it improved metabolic syndrome in animals, we put in patents for it. Now I have to add it's been very hard to get those. These are patent applications, and we have not gotten any patents, and I doubt if we will; but I have to put that up. It's very hard to get use patents as opposed to a drug patent. Allopurinol has been around for a long time, and it costs like four cents a month to take. So, I don't think I am going to make any money on this.

Alexander, Atlanta: Thank you for a fascinating presentation. I actually wanted to ask you a question. I was just identifying myself for the recording before Phil jumps on me. Would you speak to the mechanism by which fructose sets off the string of abnormalities, and particularly you indicated that oxidative stress was enhanced; and do you have insights into the intracellular mechanism?

Johnson, Aurora: Well, we now know that fructose, when you put it in a cell, stimulates oxidative stress, and that is key for activating all the pathways that lead to the proinflammatory fat accumulating step. We can show that when you put fructose in a cell, NADPH oxidase is activated, and we can block that by lowering uric acid. In addition, we have evidence that the stimulation of oxidative stress affects the mitochondria; and I am preparing a paper on this but it's through the mitochondria that it leads to fat accumulation by affecting enzymes that stimulate fat synthesis and that block fat oxidation, and the consequence is that the cell will fill up with fat.

Abboud, Iowa City: I have one more question. You mentioned that uric acid is an antioxidant. Tell me more about that.

Johnson, Aurora: So, all oxidants can be pro-oxidant or antioxidant depending upon their situation. When uric acid reacts with peroxynitrite, it will deactivate the peroxynitrite to form triuret, but in the process, it generates two radicals: triuret-carbonyl radical and an aminocarbonyl radical. We believe that in the extracellular environment, these radicals are dissipated, but intracellularly, these radicals activate processes inside the cell. We do not actually know exactly what triggers NADPH oxidase oxidation inside the cell, but we think it may be through one of these, you know, through a generation of a radical that develops as a consequence of its antioxidant or binding property. So, in other words, as it knocks out one oxidant, it generates radicals that may activate other pathways.